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# **Long term serum bile acid concentrations in 51 dogs after complete extrahepatic congenital portosystemic shunt ligation**

**Objectives:** to report the long term bile acid stimulation test results for dogs that have undergone complete suture ligation of a single congenital extrahepatic portosystemic shunt (EHCPSS)

**Methods:** data were collected from the hospital records of all dogs that had undergone a complete suture ligation of an EHCPSS (in one or two procedures). Owners were invited to return to the referral centre or their local veterinarian. A control population of dogs diagnosed with idiopathic epilepsy undergoing bile acid stimulation tests were used for comparison.

**Results:** Fifty-one study dogs were recruited. Mean  $\pm$  SD follow up time was  $62 \pm 27.4$  months. 48 dogs had no evidence of multiple acquired shunts (MAS) and a significant reduction in the pre and post-prandial serum bile acid (SBA) concentrations at long-term follow-up compared with pre-operatively, ( $p < 0.01$  and  $p < 0.01$  respectively). At long term follow-up in 64.7% and 86.3% of these dogs pre- and post-prandial SBAs were above the laboratory's reference range. Pre- and post- prandial SBAs were statistically significantly greater for dogs that had undergone a full ligation (with no evidence of MAS) at all time points compared to the control dogs ( $p < 0.001$  for all comparisons).

**Clinical significance:** SBAs do not normalise in the majority of dogs surgically corrected for their EHCPSS, remaining mildly increased. The results suggest that in dogs treated with complete suture ligation mild increases in SBAs are not clinically relevant if present in combination with no physical examination abnormalities, a normal body condition score and no relapse in clinical signs.

## **Introduction**

A congenital portosystemic shunt (CPSS) is an abnormal vascular communication that diverts blood away from the portal circulation supplying the liver into the systemic circulation. The CPSS may be intra (IHCPSS) or extra hepatic (EHCPSS) and results in liver hypoplasia and hepatic insufficiency. Clinical signs commonly include poor body condition score and signs of hepatic encephalopathy (HE) (Centre *et al.* 1990). CPSS are associated with a variety of hepatic histopathological abnormalities,

indicative of portal hypoperfusion, including biliary and arterial hyperplasia, portal vein hypoplasia and fibrosis (Cullen et al. 2006). Surgery to attenuate the shunting vessel, thus re-directing hepatic portal blood flow to the liver is the recommended treatment in most animals (Greenhalgh *et al.* 2014). The liver has a great capacity for regeneration via hyperplasia. Increased liver volume, suggestive of liver regeneration, post-surgery has been confirmed in a small number of dogs with IHCPSS and EHCPSS using contrast CT and MRI imaging (Kummeling *et al.* 2010, Stieger et al. 2007). Further studies have supported the concept that liver regeneration and angiogenesis are important in the hepatic response to CPSS attenuation (Tivers *et al.* 2015, Tivers *et al.* 2014, Tivers et al. 2014).

A variety of surgical techniques exist to achieve acute or gradual CPSS attenuation at surgery including the use of suture ligation, cellophane band, ameroid constrictor or intravascular coils. Currently there is no convincing evidence to recommend one treatment over another (Tivers *et al.* 2012). Residual shunting after the use of a gradual attenuation device is to be expected within the first few months after surgery, although this should resolve as vessel closure progresses. If attenuation is incomplete after the use of a gradual occlusion technique then clinical signs can persist. Similarly, if only a partial ligation has been performed then in some instances clinical signs can persist due to continued flow through the CPSS. Recurrence of clinical signs can occur with any attenuation method (gradual occlusion, partial or complete ligation) and can be due to the development of multiple acquired shunts (MAS) or persistent shunting through an incompletely attenuated shunt. The incidence of MAS as a complication in the published literature varies depending on the study population and/or technique used. However, the development of MAS seems to be uncommon,, with 3/29 dogs developing MAS after a partial suture ligation in one study (Burton *et al.* 2001) and at least 2/15 partial ligations and 1/13 and ameroid ring dogs developed them in another study (Winkler et al. 2003). A further study into ameroid rings found 5/112 died or were euthanized due to MAS and another study found 3/16 to have MAS following cellophane band placement (Falls et al. 2013, Landon et al. 2008). In dogs with complete shunt suture ligation and no MAS, the long term clinical outcome is considered excellent ( Bostwick *et al.* 1995, Hottinger *et al.* 1995, Hunt *et al.* 1999, Kummeling *et al.* 2004, Lawrence *et al.* 1992, Smith *et al.* 1995), but there is very limited information

on whether this excellent clinical outcome is associated with a long term return to normal biochemical and liver function (bile acid stimulation test) parameters.

Serum bile acids (SBAs) are commonly used as a diagnostic test for CPSS as well as to monitor response to surgery, due to the practical ease of performing the test and its high specificity and sensitivity (Winkler *et al.* 2014, Ruland *et al.* 2010). To the authors' knowledge there are no studies reporting long term (>18 months post-operatively) SBAs in the veterinary literature for a large group of dogs after complete shunt ligation. (Hunt *et al.* 1999; Lawrence *et al.* 1992). Hunt (1999) reported follow up at a median of 13.5 months (range 2 weeks to over 6 years) after partial or complete shunt ligation using a silk ligature, with 36 dogs having biochemistry and either a SBA sample or ammonia tolerance test (ATT) at the time of follow up. Twenty two per cent (8/36) of these were found to have abnormal ATT or post prandial SBA and seven of these dogs experienced long term problems related to persistent shunting, with 10 dogs in total experiencing relapse of clinical signs. All but one of these dogs did not undergo complete shunt ligation at surgery or imaging to investigate if they were still shunting at the time of follow up. Therefore it is unknown if the relapse in clinical signs was due to persistent shunting, the development of MAS or re-cannulisation of the originally ligated shunting vessel, all of which could have accounted for the increased ATT or SBA. A further study reported SBA values in 20 dogs at a mean time of 18.6 months (range 2.5-60) post partial ligation (9 dogs) and complete ligation (11 dogs) of a CPSS. None of these dogs had normal pre-prandial SBA values at follow-up and only four had normal post-prandial SBAs (Lawrence *et al.* 1992).

There are reports that indicate improved biochemical and SBA parameters for dogs undergoing complete shunt ligation at various short-term time points (<1yr), (Burton *et al.* 2001, Lee *et al.* 2006). However there is a lack of long term evidence on whether or not these improvements eventually normalise, stay the same or even deteriorate, and how this is related to long term clinical outcome.

The main aim of this study therefore was to investigate the long term (>18 months) biochemistry and bile acid stimulation test results for dogs that had undergone complete suture ligation of a single congenital extrahepatic portosystemic shunt (EHCPSS). Our null hypothesis was that there would be

no difference in biochemistry and bile acid stimulation test results between dogs that had undergone complete suture ligation of a single congenital extrahepatic portosystemic shunt (EHCPSS) and control dogs free of liver disease. We hypothesised that these results would be normal at this length of time post-operatively for completely attenuated EHCPSS, without evidence of MAS, due to blood flow through the shunt being completely occluded and the substantial regenerative capacity of the liver following surgical correction. Additionally, we aimed to assess the long term physical examination, body condition score and abdominal ultrasound findings for these dogs to accurately document their clinical outcome following complete shunt ligation.

## **Materials and Methods**

Institutional ethical approval was obtained for the study. Medical records were reviewed for all dogs that had undergone ligation of a single EHCPSS between January 2000-February 2012. During the study period at the authors' institution, complete attenuation with polypropylene (Prolene)<sup>a</sup> was performed on all dogs with CPSS if tolerated based on subjective and objective parameters (including measurement of intraoperative portal pressures, a portovenogram and subjective assessment of intestinal and pancreatic colour). In dogs that could only tolerate partial shunt ligation at the initial surgery, the shunt was partially attenuated as much as was deemed appropriate based on the above parameters using a polypropylene ligature. A repeat surgery was recommended for all dogs, regardless of clinical response, approximately three months later with the aim of achieving complete shunt ligation in a second procedure. Those dogs that did not return for a second surgery were excluded from the study.

Data collected included signalment, body condition score (BCS), surgery date, anatomical description of the CPSS (at the time of first surgery for those that went on to have two surgeries) and whether a complete or partial ligation was achieved in one or two surgical procedures. Each dog's history was reviewed and serum bile acids, complete blood count and serum biochemistry from pre- operatively

(at the time of first surgery) and at short term follow up (three months post-operatively from when a complete attenuation had been achieved) performed at the referral centre were recorded.

Long term (>18 month) follow up data was collected by initially contacting referring veterinary surgeons to ascertain if the animal was still alive and to obtain up to date client information and clinical histories. For those alive, owners were contacted by telephone, letter or email and invited to participate in the study.

Owners were instructed not to feed animals after 10pm the night prior to the appointment. The same veterinary surgeon took a current history, including diet, medications and health problems since the last visit, and performed a physical examination and BCS,. A blood sample was taken for pre-prandial serum bile acids (SBA) and biochemistry. Dogs were then fed and a blood sample for post-prandial SBA was taken two hours later. In the latter half of the study an abdominal ultrasound was performed by one of three board certified radiologists. For owners unable to return to our hospital, biochemistry and bile acid stimulation tests were offered via the referring practice. As part of a related but separate study, health-related quality of life questionnaires (HRQoL) were also completed by owners (X et al. Submitted).

Blood samples were sent to Idexx Laboratories for serum bile acid concentration and biochemistry analysis. The laboratory's reference ranges are 0.1-5.0 $\mu$ mol/L and 0.1-10.0 $\mu$ mol/L for pre- and post-prandial samples respectively. Albumin, alanine transferase (ALT) and alkaline phosphate (ALP) were analysed from the biochemical results. The laboratory's reference ranges for these are albumin 25-40 g/l, ALT 5-60 U/L and ALP  $\leq$ 130 U/L.

A control population of "normal" dogs undergoing bile acid stimulation tests and taken from a previous unrelated study performed at the authors' institution were used for comparison. The control samples were obtained as part of the diagnostic investigation for idiopathic epilepsy in accordance with tier 2 of the International veterinary epilepsy task force consensus (De Risio et al. 2015).

Statistical analysis was performed using a commercially available software package (SPSS IBM Statistics version 20). Categorical data were reported as percentages and compared with Chi-square or

Fisher's exact tests. Continuous data were assessed for normality using the Shapiro-Wilk Test and histograms. Median and 25th-75th percentiles were reported for skewed data and mean  $\pm$  standard deviation were reported for normally distributed data. Normally distributed continuous data were analysed using an independent samples t-test and skewed data were analysed with a Mann-Whitney U test for independent samples or Wilcoxon signed rank test for related-samples. Significance was set at  $p < 0.05$ .

## Results

One hundred and forty five dogs met the inclusion criteria. Of these 94 (66.2%) were still alive at a mean  $\pm$  SD follow up time of  $177 \pm 75$  months. Sixteen dogs (11.3%) had died of an unrelated cause at a mean time of 194 months post-operatively (SD 71). Two (1.4%) had died of causes related to CPSS, 13 and 201 months post-operatively. Six (4.2%) dogs died of an unknown cause at a mean time of 212 months post-operatively, (SD 124). Twenty five (17.6%) dogs were lost to follow up at a mean time of 263 months post-operatively (SD 75).

Fifty-one dogs participated in the study with three of these having their follow up blood work performed by their local veterinary practice. The mean  $\pm$  SD follow up time for these cases was  $62 \pm 27.4$  months from time of complete shunt ligation.

At first surgery, 49% (n=25) of dogs had a full attenuation, and 51% (n=26) had a partial attenuation. Of the 27 dogs that had a second surgical attenuation, 26 tolerated a full attenuation, and one dog had developed MAS but had no further shunting through the original CPSS as seen via portovenography.

A variety of breeds were represented with Miniature Schnauzers (15.4%) and cross breeds (13.5%) being the most common, (table 1). Sex and neutering status are presented in table 2. The median age at first surgery was 11.9 months (5.8-19.5).

## Physical examination

None of the dogs examined (n=48), had physical examination findings potentially associated with a CPSS. Twelve dogs had abnormalities including periodontal disease (n=3), ocular discharge (n=2), lipoma (n=2), and one each of cyst on neck, bilateral luxating patella, pyoderma, brachycephalic obstructive airway syndrome and grade II/VI heart murmur. The median body condition score was 5/10 (range 4-6/10). Pre-operative body condition score was only available for three dogs and therefore no comparison was made. No owners reported a relapse in clinical signs since surgery.

## Complete attenuation dogs, n=48

### *Bile acid stimulation test results*

The median pre-operative pre- and post-prandial SBA were 99.5 umol/L (62.3-124.8) and 245 umol/L, (147.7-351.2) respectively (results available for 45 dogs).

At short term follow up (mean 12 months, SD 15), they were a median of 7.0 umol/L (1.28-19.65) and 21.6 umol/L (14.9-47) respectively, (results available for 22 dogs).

At long term follow up (mean  $\pm$  SD 158  $\pm$  832.7 months) they were a median of 14.5 umol/L (1.52-29.5) and 38.9 umol/L (17.1-52.5) respectively, (n=48 dogs).

Pre-operatively, 98% of pre and 100% of post-prandial bile acids were above the laboratory reference range. At short term follow up 82.4% pre- and 98% of post- were above the reference range. At long term follow up these numbers were 64.7% and 86.3% for pre and post-prandial respectively.

There was a significant reduction in the pre and post-prandial serum bile acid concentrations at both short ( $p < 0.01$  and  $p < 0.01$  respectively) and long term follow up, ( $p < 0.01$  and  $p < 0.01$  respectively, figure 1 and 2), compared to the pre-operative values. There was no significant difference in pre- or post- prandial serum bile acid concentrations between short and long term follow up ( $p = 0.09$  and  $p = 0.06$  respectively, figure 1 and 2).



178

179 *CBC and biochemistry results*

180 At long term follow up, two dogs were hypoalbuminaemic (15.8 and 27.9 g/l) and seven dogs had  
181 increased ALT at a median of 179.3 U/l. No other abnormalities were detected.

182 There was a significant difference in the PCV between pre-surgical and short term values, increasing  
183 from a mean of 40.4% (range 38.4-50.6%) pre-operatively to 48.2% (45.7-50.3%) at short term,  
184 ( $p=0.016$ ). There was insufficient data available to assess PCV at long term follow up. There was a  
185 significant decrease in ALP values between pre-surgical and 3 month post-surgical samples ( $p=0.004$ )  
186 and between pre-surgical to long term ( $p<0.001$ ), but no difference between short and long term  
187 follow up ( $p=0.45$ ). There was a significant decrease in ALT values between pre-surgical and 3 month  
188 post-surgical samples ( $p=0.002$ ) and between pre-surgical and long term follow up samples  
189 ( $p<0.001$ ). However the ALT did increase significantly between 3 month post-surgical and long term  
190 follow up samples ( $p=0.04$ ).

191 There was a significant increase in urea, creatinine and albumin values between pre-surgical and short  
192 term samples ( $p<0.001$  for all) and for all values between pre-operatively and long term ( $p<0.001$   
193 for all). There was no significant difference in urea or creatinine values between 3 month post-  
194 surgical and long term follow up samples ( $p=0.45$  and  $p=0.91$  respectively), but there was a  
195 significant increase in albumin values from 3 month post-surgical to long term follow up samples  
196 ( $p=0.017$ ).

197

198 *Abdominal ultrasound results*

199 Overall 27 dogs underwent abdominal ultrasound at median follow up of 1406 days (range 598-2917),  
200 25 of which were in the complete attenuation group. These 25 dogs had no evidence of persistent  
201 shunting or MAS. Two dogs had evidence of urolithiasis and one of these dogs had a subjectively  
202 small liver.

203

Four of 48 dogs had a post-prandial bile acid at long term follow up > 100 umol/L. Of these four dogs, three had an abdominal ultrasound performed; in one dog no evidence of liver abnormalities, MAS or persistent shunting was seen but some cystoliths were present. A second dog had situs inversus and a full abdominal ultrasound could not be performed due to lack of visualisation, however a subjectively small liver and cystoliths were seen (as described above). A third case had some turbulence associated with the shunt ligation site, as had been visualised on a previous post-operative ultrasound, however this dog had undergone an exploratory laparotomy and portovenogram where no evidence of persistent shunting or MAS were seen. The final case was not able to come back to our referral centre for an abdominal ultrasound due to owner restrictions.

At short term follow up, excluding the two cases described above where an abdominal ultrasound was not or could not be performed, the pre-prandial 95<sup>th</sup> percentile was 49.2 umol/L and post-prandial 90.6 umol/L. At long term follow up these values were 93.9 umol/L and 119 umol/L respectively.

### **Multiple acquired shunt dogs, n=3**

Three dogs were diagnosed with multiple acquired shunts (MAS). One dog was diagnosed at the time of second surgery, where the previous macroscopic shunt had closed despite being partially attenuated with a polypropylene ligature. The remaining two dogs were found to have MAS on abdominal ultrasound when re-examined for this study, following full shunt attenuation. This population consisted of two Cocker Spaniels one male castrated and one female spayed (age 4.8 and 9.8 years respectively), and one male castrated Bichon Frise, age 3.6 years.

Two dogs had a pre-operative pre-prandial SBA value available (125.4 and 165 umol/L). All dogs had a pre-operative post-prandial sample (90, 307.3 and 338.9 umol/L). At short term follow up (n=3), pre-prandial SBAs were 1.4, 10.7 and 17.3 umol/L and post-prandial SBAs were 23.4, 92 and 133 umol/L. At long term follow up, (mean 124 months, SD 47.8), the pre-prandial SBAs were 50.6, 102.4 and 199.2 umol/L and post-prandial 98.4, 147.3 and 224.7 umol/L. All dogs had SBAs above

the reference range pre-operatively and at long term follow up. The number of dogs in this group was too small to perform statistical comparisons.

On biochemical analysis one dog had increased ALT activity (66.2 U/L). No other abnormalities were detected. The three dogs with MAS were all receiving a restricted protein diet and one was receiving lactulose. No owners reported a relapse in clinical signs or other abnormalities and the only abnormality detected on physical examination was ventral pyoderma in one dog.

### **Control dogs (n=36)**

Median age of the control dogs was 19 months (range 6-165 months). Breeds are presented in table 3. The median pre-prandial SBAs for control dogs were 0.9 umol/L (25th-75th percentiles 0.5-1.5) and post-prandial 3.5 umol/L (25th-75th percentiles 1.8-5.3). Pre- and post- prandial SBAs were statistically significantly greater for dogs that had undergone a full attenuation with no evidence of MAS at all time points compared to the control dogs ( $p < 0.001$  for all comparisons).

### **Discussion**

The clinical outcome of all dogs available for long term follow-up following complete CPSS ligation surgery was excellent based on the body condition score and lack of physical examination abnormalities. However, our null hypothesis that the SBA of dogs following complete surgical ligation of a CPSS would have returned to normal at long term follow up was rejected. There was a statistically significant decrease in pre and post-prandial SBA at long term follow up compared to pre-operatively, but 65% and 83% of pre- and post-prandial SBA respectively were still above the laboratory reference range at a mean follow up of 62 months. This is an interesting finding and the reason(s) for it are unclear. Abdominal ultrasound was performed in the latter half of the study (once extra funding became available to do this) to evaluate dogs for possible development of MAS that were not causing clinical signs as a reason for increased SBA. Only two dogs (out of 27) were

detected with MAS on abdominal ultrasound at long term follow-up. However the dog with the greatest post-prandial SBA value (215  $\mu\text{mol/L}$ ) was unable to return for an abdominal ultrasound, and the dog with the second greatest value was not able to have a full ultrasound performed due to her situs inversus. Interestingly this dog did have a subjectively small liver and cystoliths and therefore it would be reasonable to suspect either development of MAS or persistent shunting. However this dog had a full suture ligation, making the latter possibility unlikely. The sensitivity and specificity of abdominal ultrasound for the detection of MAS has not been studied extensively although in one study 1/6 cases of MAS were detected by AUS compared to 4/5 with computed tomography angiography (CTA), (Kim *et al.* 2013). CTA would have been a more sensitive and specific method of detecting MAS. However it is more expensive and requires sedation. Regardless, the incidence of MAS detected in this study is comparable with many other studies that report rates of MAS between 10-18% (Burton *et al.* 2001, Winkler *et al.* 2003, Landon *et al.* 2008). Most importantly, the rate of MAS in this study (3.5%) is simply far too low to be solely responsible for the 56% and 79% persistently elevated pre- and postprandial SBA at long term follow-up, even if the imaging methods for detecting MAS are not 100% sensitive. Additional support for MAS not being a major cause of the increased SBAs in our population of dogs was provided by the three dogs that were known to already have MAS; none of these dogs with MAS had a post-prandial SBA  $< 98 \mu\text{mol/L}$ . Overall, our study showed that long term, pre-prandial values above 94  $\mu\text{mol/L}$  and post-prandial above 119  $\mu\text{mol/L}$  give a 95% chance of being abnormal i.e. further investigation of likely residual shunting is warranted.

Continued shunting through the original CPSS is another possibility for persistently increased SBA if the shunt has not been completely attenuated (by a ligature, cellophane band or ameroid constrictor). However, the dogs in this study had documented complete shunt ligation at surgery eliminating residual shunting as a cause for elevated SBA seen in this population of dogs.

We therefore conclude that there must be another cause for the long term increased SBAs seen in the majority of dogs receiving a complete EHCPSS ligation in this study, and assume that subclinical abnormal liver pathology persists once the CPSS is ligated and normal hepatic portal flow resumes.

Aetiologies for this persistent abnormal liver pathology include concurrent microvascular dysplasia / primary portal vein hypoplasia (PVH) and/or a failure of the liver to recover normal function after correction of the abnormal blood flow through the CPSS. PVH has been reported as a separate entity in dogs and it has also been suggested that it can occur concurrently with a macroscopic shunt (Allen *et al.* 1999, Christiansen *et al.* 2000, Phillips 1996, Schermerhorn *et al.* 1996, ). Our data suggests that there is a spectrum of varying degrees of concurrent congenital microvascular dysplasia that results in a high proportion of dogs with a CPSS failing to have normal SBAs after surgical correction of the single congenital macroscopic shunt. A similar suggestion was made in a previous study where the authors suggested that it may be possible for some dogs with EHCPSS to have microscopic shunting as well as a macroscopic shunt, resulting in failure of SBAs to normalise following successful surgical ligation (O'Leary *et al.* 2013). It would be very difficult to provide evidence for this theory as the histopathological hepatic findings associated with PVH are indistinguishable from those seen in dogs with a macroscopic CPSS and all of the dogs in our study had an excellent long term clinical outcome. We therefore conclude that whatever the exact aetiology of the liver pathology causing persistent, mildly (<50 umol/L) increased SBAs in dogs with complete CPSS ligation but without MAS (median pre prandial 14.5 and post prandial 39.5 umol/L), it is of minimal clinical significance with regards to long term outcome and does not require further imaging investigations, medical therapy or special diet. This is further supported by findings in a concurrent study performed at our hospital, assessing long term quality of life following surgical attenuation of an EHCPSS or IHCPSS (X *et al.* Submitted). Additionally, the 95% confidence intervals calculated in the current study suggest that dogs with pre-prandial and post-prandial values approximately higher than 94 umol/L and 119 umol/L respectively do warrant further investigation of likely persistent shunting. Interestingly, people with liver disease can suffer from minimal hepatic encephalopathy (MHE) *et al* 2007) and although they do not show obvious signs of hepatic encephalopathy (HE) they do have significant abnormalities in neurophysiological performance and on psychometric testing which may be something too subtle for us to detect at this stage in dogs.

We will continue to recommend a repeat SBA stimulation test at short term follow up post-operatively so that if the bile acids are moderately or markedly increased an abdominal ultrasound examination or other imaging can be performed to assess for residual shunting or MAS. However, as there was no significant difference between the SBA at short term and long term follow up, repeat SBA testing seems unnecessary in the long term providing the dog remains free of clinical signs.

The main limitation of our study is the small number of dogs with MAS, allowing no statistical comparison between groups. We suspect that if a greater number of dogs had been included with MAS, a statistically significant difference would have been found in both pre- and post-prandial bile acids at long term follow up. However the limited numbers also reflects the low number of dogs that develop MAS following full ligation with a prolene suture.

Of further note, the few papers in the veterinary literature that do include results of long term follow-up SBAs have either no reference range reported making it hard to make comparisons between papers on the percentage of dogs with abnormal results, or variable reference ranges from 5.3-9.9 umol/L up to <40umol/L (Burton *et al.* 2001, Hunt *et al.* 1999, Lawrence *et al.* 1992, O'Leary *et al.* 2014, Winkler *et al.* 2014).

## **Conclusion**

SBAs do not normalise in the majority of dogs surgically corrected for their EHCPSS although they become mildly rather than moderately or severely increased in the vast majority of dogs. Given the excellent clinical outcome of all dogs in this study, long term mildly increased SBA is not a major concern if present in combination with no physical examination abnormalities, a normal body condition score and no relapse in clinical signs.

## Figure Legends

**Figure 1:** Pre-prandial serum bile acids for dogs with an extrahepatic congenital portosystemic shunt (EHCPSS) pre-operatively and at short and long term follow-up after complete shunt ligation. The horizontal line in the box represents the median. The box represents the interquartile range (25th to 75th percentiles). The whiskers represent 1.5 times the interquartile range. Open circles represent outliers, stars represent extreme values. The dashed line shows the upper end of the laboratory reference range (5.0umol/l).

**Figure 2:** Post-prandial serum bile acids for dogs with an extrahepatic congenital portosystemic shunt (EHCPSS) pre-operatively and at short and long term follow-up after complete shunt ligation. The horizontal line in the box represents the median. The box represents the interquartile range (25th to 75th percentiles). The whiskers represent 1.5 times the interquartile range. Open circles represent outliers, stars represent extreme values. The dashed line shows the upper end of the laboratory reference range (10.0umol/l).

No conflicts of interest have been declared

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